Home Office

NON-TECHNICAL SUMMARY

Protecting Hearing and Vestibular Systems.

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

hearing, vestibular, hair cell, deafness, balance disorders

Animal types	Life stages
Zebra fish (Danio rerio)	Embryo and egg, Neonate, Juvenile, Adult, Aged animal

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aims of this project are to reduce, protect and/or alleviate hearing loss and balance disorders caused by aging, genetics or the environment by identifying new routes and/or compounds for therapeutic intervention

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Our inner ear, located behind the eardrum, comprises two parts: the cochlea and the vestibular system. Our cochlea detects vibrations made by sound, translating these into electrical signals our brain perceives as noise, while our vestibular system detects body movement; translating these into signals our brain uses to maintain balance and orientation. Both structures contain hair cells, specialised cells with height ranked hair-like projections on the top end of the cell that are deflected by sound and movement transferring this movement into electrical signals. In addition to the hair cells, there are specialised gelatinous accessory structures that either transmit the sound waves and body movements to the hair-like projections (stereocilia) or act as structures against which the projections can push. Defects in the hair cells and the accessory structures can result in deafness and/or balance disorders.

The hair cells of the inner ear are lost with age; as a result of disease; and due to environmental conditions, such as excessive noise or as an unwanted side effect of certain life-saving medication. It is estimated that 40% of people over the age of 50 have hearing loss. While one in 2000 babies is born deaf due to one of a number of genetic defects.

Vestibular disorders, like hearing loss, can again result from age; or as an unwanted side effect of certain life- saving medication but these are less well understood as they can, to some degree, be compensated for by the visual system. As we age, however, our eyesight also fails. It is suggested that defects in the vestibular system may underlie balance problems in the elderly with the NHS estimating that falls cost the taxpayer more than £2.3 billion per year.

The proposed research will use zebrafish as a model system to understand hearing loss and balance disorders and to discover new and refine existing compounds that will protect human hearing and balance. In using zebrafish as a model we can also aim to answer fundamental questions in fish biology regarding the structure and function of the lateral line compared to the inner ear. The lateral line is a series of discrete sensory patches running along the fish's body that sense vibrations and pressure changes in the surrounding water. These sensory patches have hair cells and accessory structures similar to those in the mammalian inner ear, though in the fish they play an important role in shoaling, prey avoidance and have overlapping functions with the inner ear in orientation. Studies have shown that damage to the lateral line results in fewer fish swimming in the same direction and collisions between neighbours increasing resulting in physical damage and the risk of infection.

What outputs do you think you will see at the end of this project?

The outputs at the end of this project will include new information that would lead to publications or future research proposals. This information will help us to better understand 1) how some clinically important medications cause the loss of hair cells from the inner ear, leading to deafness and balance disorders, and identify compounds (products) that could protect these hair cells 2) how either age of mutations in certain genes leads to defects in balance and orientation.

Who or what will benefit from these outputs, and how?

In the short term (2-3 years) this project will advance our understanding of how certain medications and mutations in our genes cause deafness and balance disorders benefiting the research community. This research may also lead to the discovery of drugs and strategies for preventing and/or managing hearing loss and balance disorders benefiting people at risk of these conditions (5+ years). Balance disorders are particularly under researched with few viable interventions for those who experience them, therefore this research will be beneficial to other researchers (2-3 years) and people experiencing balance disorders (5+ years)

In addition to the benefit this will have for humans this research will also benefit fisheries research as it will improve our understanding of the genes responsible for balance and orientation in fish and the roles played by the inner ear and lateral line in shoaling, prey avoidance and orientation. This information could be used by researchers to improve fish welfare in commercial fisheries and/or aquaria.

How will you look to maximise the outputs of this work?

Our data will be readily available to the research community. We maintain regular communication with the research community in the UK with meetings, collaborations and email correspondence with other hearing researchers, as well as zebrafish researchers and technicians to exchange good practice in animal care and welfare. We attend and present data at meetings with ENT clinicians and audiologists to exchange information on the relevance and need for research in human health it is these discussions that have led to our research into balance disorders. We endeavour to disseminate our research at relevant international meetings such as the Midwinter Research Meeting of the Association for Research in Otolaryngology in the USA, the largest scientific meeting in hearing research, Inner Ear Biology meeting and relevant zebrafish meetings. We will publish in open-access journals or traditional journals that offer the option of making content freely available. In addition to this we will present at public engagement and stakeholder events, for example RNID organise events for donors and people with hearing loss to meet the scientists and discuss their research we have presented these several times.

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 18800

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

This research project will use zebrafish as the model species. Zebrafish provide advantages over mammals as many of the experiments can be done on larvae under the protected age as the hair cells and accessory structures are formed and fully functional early in development. In addition, this project seeks to understand the contribution of the inner ear and lateral line (unique to fish and amphibians) to hearing and balance in fish therefore zebrafish make an ideal model system. Protocols conducted on the protected life stages are the production and maintenance of genetically modified animals and also behavioural tests. Non-animal alternatives are not possible, as functional hair-cell lines do not yet exist. The literature has some examples of organoids (organ-like structures derived from stem cells) and mouse cell lines derived from hair cells but these do not respond well to sound-like stimulation and are less sensitive to drugs that cause hearing loss compared to fully functional hair cells. In addition to this some of the research in this project seeks to investigate how altering hearing and balance genes effect animal behaviour, this cannot yet be done using synthetic methods, particularly given that balance disorders are multifaceted and can to some extent be compensated for by environmental cues.

Typically, what will be done to an animal used in your project?

Protocols conducted on the protected life stages are the production and maintenance of genetically modified animals and behavioural tests.

Animals involved in the production and maintenance of genetically modified animals will be naturally mated to produce larvae for experiments (before protected life stages) or larvae, juveniles and adults for behavioural experiments. Animals may also have small amounts of tissue removed for genotyping or in the unlikely event that natural mating is not possible be used for production of gametes for in vitro fertilisation.

Behavioural tests will involve wild type or genetically modified animals being placed in an observation chamber and videoed free swimming, swimming in a current (assessing rheotaxis) or when stimulated (assessing the startle response). The time in the observation chamber will not exceed an hour. Animals may undergo this procedure up to 10 times in their lifetime to understand how behaviour changes with age.

What are the expected impacts and/or adverse effects for the animals during your project?

The majority of the animals on this project are expected to show no adverse effects. Those animals with mutations in hearing and/or balance genes may show abnormal behaviour, for example occasional spinning or circling.. This may occur throughout the life of the animal though it is possible some of the animals may adapt and show no abnormal behaviour. To minimise any distress caused to the fish we have developed a number of mitigations. As defects in the vestibular system can be largely compensated for by the visual system mutant fish showing abnormal behaviour are housed with wild type companion fish to aid in shoaling, are provided with appropriate environmental enrichment (e.g.

plastic plants and or housing/refuges) and also provided with tank adornments that simulate the river bottom allowing the animal to calculate tank depth and therefore aid in orientation.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The expected severity of the majority of animals for both the production and maintenance of genetically modified animals and behavioural tests is expected to be either mild (max 50-55%) of subthreshold (min 25%). Some moderate phenotypes are expected in approximately 20-25% of cases of those animals with mutations in hearing and/or balance genes. .

What will happen to animals used in this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Non-animal alternatives are not possible, as fully functional hair-cell cell culture lines do not yet exist. In addition to this some of the research proposed seeks to investigate how defects in hearing and balance genes effect animal behaviour which cannot yet be done using synthetic methods.

Which non-animal alternatives did you consider for use in this project?

We have looked in the literature and considered organoids (organ-like structures derived from stem cells) and mouse cell lines derived from hair cells.

Why were they not suitable?

Organoids and mouse cell lines are not suitable as these do not respond well to sound-like stimulation and are less sensitive to drugs that cause hearing loss compared to fully functional hair cells.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific

objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Numbers are based on actual numbers from similar zebrafish experiments conducted on a previous project licence with adjustments made for the increase in the number of anticipated behavioural tests, genetic variability, and environmental variability. The number of animals required for production of GA lines varies greatly depending on the gene mutated or the DNA inserted. In addition, pilot data suggests that not all homozygous animals will show the phenotype as it is anticipated that some animals may adapt to vestibular defects and therefore a larger number may be needed.

We expect to use up to 3000 fish for the generation of 40 new strains. This is calculated based on the assumption that for 25 strains only 1% of the animals will be able to transmit inserted DNA to the next generation, for the other 15 strains we calculated a 5% success rate of producing a desirable gene mutation able to be transmitted to the next generation.

We expect to use up to 10,000 GA fish for breeding purposes to supply embryos for experiments and for maintenance of the strains. This is based on 50 different strains, the addition 10 being combinations of strains. We also expect to use 5,000 of those 10,000 fish for testing vestibular function and rheotaxis.

Zebrafish are shoaling animals and would ideally be housed in groups of 10-20. New generations are raised when adults are between 6-10 months the optimal age for successful reproduction. Older generations are also required for aging experiments. For some combinations of strains, only a small proportion of the fish are the correct genotype. Over a 5 year period, this adds up to 10,000 fish. A further 800 fish will be used for the production of gametes.

The calculation of 18800 fish is the maximum number of fish used for all experiments the number is actually lower as the 5000 fish used in behavioural experiments and 800 for production of gametes are the continued use of the GA fish.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The experiments were designed in consultation with a statistician and lecturer in experimental design who is familiar with the research. A calculation of the number of animals needed is based on pilot data from a previous project licence analysed by the statistician.

We can preserve sperm to reduce live animals, negating the need to recreate strains or combinations of strains, and reduce genetic drift.

We aim to trial genotyping embryos < 5dpf using either the protocol outlined in Wilkinson et al 2018 (https://doi.org/10.2144/000114509) or the Zebrafish Embryonic Genotyper, which gently removes cells from the skin for genotyping. If these are successful then only fish of the required genotype will then be raised to adulthood, reducing numbers.

Finally, as these experiments use zebrafish as a model, we have the opportunity to answer questions on the role played by the lateral line and inner ear in maintaining balance and orientation in fish, with only a few extra experiments as we can use data from the inner ear mutants produced in other experiments.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Pilot studies have been undertaken on a previous licence. These pilot studies were conducted using zebrafish and mice with a mutation in a gene expressed in the vestibular system of the inner ear in both animals. This data showed that only 20-25% of animals showed an adverse phenotype.

To keep the number of fish to a minimum the GA zebrafish used for breeding will also be used for behavioural experiments. Optimisation of experiments will mean that tissue will be taken from existing experimental animals so that no additional animals will be needed for tissue collection e.g. animals no longer needed for breeding and/or behavioural experiments will be culled and tissue used for analysis of morphology.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This research project will use zebrafish as the model species with the majority of the experiments done on larvae under the protected age as the hair cells and accessory structures are formed and fully functional early in development. Protocols conducted on the protected life stages are the production and maintenance of genetically modified animals and also behavioural tests e.g. videoing zebrafish in a viewing chamber both free swimming and in a current, testing alertness (startle reflex).

Why can't you use animals that are less sentient?

Production and maintenance of genetically modified animals must be done on fish that have reached sexual maturity. We will be using a number of life stages to conduct behavioural assays to understand how balance changes with age both for human health benefits and fish welfare therefore other less sentient species or terminally anaesthetised animals can't be used.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

To minimise any distress caused to the fish by the procedures we have developed a number of mitigations. As defects in the vestibular system can be largely compensated for by the visual system mutant fish showing a moderate phenotype are housed with wild type companion fish to aid in shoaling, are provided with appropriate environmental enrichment (e.g. plastic plants and or housing/refuges) and also provided with tank adornments that simulate the river bottom allowing the animal to calculate tank depth and therefore aid in orientation.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

I will follow the RSPCA Guidance on the housing and care of zebrafish, the NC3R refining procedures documentation on their website and also check the Norecopa website as they publish links to articles showing best practice and refinements for animal welfare.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I am on the NC3R mailing list and have attended relevant workshops. If possible I attend zebrafish husbandry association meetings, if not possible I discuss the meeting with colleagues who have attended. I am in regular contact with the NACWO who keeps me updated of any advances and we discuss how we implement them.